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# Multiple Splicing Variants of Two New Human ATP-Binding Cassette Transporters, ABCC11 and ABCC12

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**Two new human ABC transporters, ABCC11 and ABCC12, were cloned from a cDNA library of human adult liver. ABCC11 and ABCC12 genes consist of 30 and 29 exons, respectively, and they are tandemly located in a tail-to-head orientation on human chromosome 16q12.1. The predicted amino acid sequences of both gene products show a high similarity with ABCC5. The transcripts of ABCC11 and ABCC12 genes were detected by PCR in various adult human tissues, including liver, lung, and kidney, and also in several fetal tissues. By searching cDNA libraries from various human tissues, we have identified alternative splicing variants of ABCC11 and ABCC12 genes at significantly high frequencies. One splice variant lacking the exon 28 corresponded to about 25% of total ABCC11 gene transcripts. Furthermore, four splicing variants encoding putatively short peptides were predominant in ABCC12 gene transcripts. Those splicing variants may represent diverse biological functions of these ABC transporter genes.** © 2001 Academic Press

*Key Words:* **ABC transporter; ABCC11; ABCC12; genetic polymorphism; alternative splicing; human chromosome 16.**

The ATP-binding cassette (ABC) transporters form one of the largest protein families and play a biologically important role as membrane transporters or ion channel modulators (1, 2). Until now more than 48 human ABC-transporter genes have been identified and sequenced (3, 4). Based on the arrangement of molecular structure components, i.e., the nucleotide binding domain and the topology of transmembrane domains, human ABC transporters are classified into seven different gene families (A to G) (2–4). Mutations of human ABC transporter genes have been reported to cause of certain genetic diseases, such as Tangier disease (5–7), cystic fibrosis (8), Dubin–Johnson syndrome (9), Stargardt disease (10), and sitosterolemia (11).

The ABCC gene family (according to the new nomenclature of human ABC transporter genes) comprises the members of multidrug resistance-associated proteins (MRP) (12), sulfonylurea receptors (SUR) (13), and cystic fibrosis transmembrane conductance regulator (CFTR) (8). MRP1 (ABCC1 according to new nomenclature for human ABC transporter genes) was first identified by molecular cloning from human multidrug-resistant lung cancer cells (14). MRP1 encodes one of the previously characterized GS-X pumps (15) that transport leukotriene $C_4$ (16) and drugs either conjugated with glutathione (GSH), glucuronide or sulfate (17). In addition, MRP1 reportedly transports some anticancer drugs in an unmodified form together with GSH (18, 19). After the discovery of the MRP1 gene, six MRP1 homologues have been identified. At present, the human MRP subfamily consists of at least seven members (MRP1, MRP2/cMOAT, MRP3, MRP4, MRP5, MRP6, and MRP7) (2, 3, 12, 20, 21) and exhibits a wide spectrum of biological functions. Accumulating evidence shows that ABC transporters of the MRP subfamily are involved in transport of drugs as well as endogenous substances (12, 16–19, 22, 23).

The draft sequence of the human genome has recently published (24, 25), and more than 50 of human ABC transporter genes have been anticipated to exist in the human genome (4). However, at present, because of the difficulty in the precise prediction of exon–intron boundaries using currently available software programs, actual cloning and sequencing of cDNA is still a critical step for our understanding of the molecular

The cDNA sequences of ABCC11 and its transcript variant A as well as ABCC12 variants A, B, C and D have been registered in GenBank under the Accession Nos. AF367202, AF411579, AF395908, AF395909, AF411577, and AF411578, respectively.

Abbreviations used: ABC, ATP-binding cassette; MRP, multidrug resistance-associated protein; PCR, polymerase chain reaction; GS-X pump, ATP-dependent glutathione S-conjugate export pump.

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structure and function of novel ABC transporters. We have recently discovered two novel ABC transporters, i.e., human ABCC11 and ABCC12 that belong to the ABCC gene family and are located on the chromosome 16q12.1. In the present study, we have analyzed multiple splicing variants transcribed from ABCC11 and ABCC12 genes and herein demonstrate the gene features and expression profiles of these two ABC transporter genes in human organs.

## MATERIALS AND METHODS

*Cloning of cDNA encoding human ABCC11 and ABCC12 and their splicing variants.* The draft sequence of the human chromosome 16 (GenBank Accession No. AC007600) was analyzed using the GENSCAN program (http://genes.mit.edu/GENSCAN.html) to predict exons. EST clones were extracted from the currently available EST database to find partial sequences of ABCC11 and ABCC12.

To clone full length and splicing variant of ABCC11 cDNA, the following three sets of PCR primers were designed: the 5′-part (C11-1 forward primer: 5′-ATGGCTTCGCGCTGCTCTCT-3′ and C11-1 backward primer: 5′-CCTCAGATGTGTGATGCCGAGCCTT-3′), the middle part (C11-2 forward primer: 5′-GGTATTCATGACAAGAATGG-3′, and C11-2 backward primer: 5′-GACGATCAGCACCACGAAGA-3′), and the 3′-part (C11-3 forward primer: 5′-CCTTGA-GTTGGAGGGTCTAC-3′, C11-3 backward primer: 5′-AAGTAGCCTATTCCAGGGTTT-3′). PCR was performed using human adult liver cDNA (Clontech, Palo Alto, CA) and the Ex Taq polymerase (Takara, Japan), where the PCR consisted of 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 2 min. In addition, the 3′-portion of noncoding region was cloned by 3′-rapid amplification of cDNA ends (3′RACE) using human liver Marathon-Ready cDNA (Clontech) and two primers (first primer: 5′-GCCAGGG-CTGTGCTTCGCAAC-3′ and nested primer: 5′-CAGGGCTGCAC-CGTGCTCGT-3′) under the PCR conditions of 30 cycles of 94°C for 1 min and 68°C for 2 min.

To clone four splicing variants of ABCC12 cDNA in a similar manner, six sets of PCR primers were designed: the 5′-parts (C12-1 forward primer: 5′-ATCAGGATGGTGGGTGAAGG-3′, C12-1 backward primer: 5′-CTGGCTTCATGCTCCCATGTC–3′ and C12-2 forward primer: 5′-GGTGGGTGAAGGACCCTA–3′, C12-2 backward primer 5′-CAGAACCGATTTGAG GCTGTCACT-3′), the middle parts (C12-3 forward primer: 5′-TGAAGCCAGC-AGGAAAGTACC-3′, C12-3 backward primer: 5′-CTGCAGAAA GTTCTCTGCGT–3′ and C12-4 forward primer 5′-CTCCTCTCTGCATGACACGG–3′, C12-4 backward primer 5′-CACACA-AAGCAGCTGACGTTC-3′), the 3′-parts (C12-5 forward primer: 5′-GTAAGGTACAACTT-GGATCCCT-3′, C12-5 backward primer: 5′-TGCTGCTAGTAACATCGCAA-3′ and C12-6 forward primer: 5′-CACCGCCTCTATG GACTCCAAGACTG-3′, C12-6 backward primer: 5′-CGCTACAAATCTGTGTCATTACCAC-3′). PCR was performed using the human adult liver, pancreas and testis cDNA (Clontech). The PCR consisted of 35 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 2 min.

The sequences of PCR products were analyzed by automated DNA sequencing (TOYOBO Gene Analysis, Japan). The whole cDNA sequences of ABCC11 and ABCC12 as well as their splicing variants were determined by assembling the partial sequences thus obtained. The cDNA sequences of ABCC11 and its splicing variant A as well as ABCC12 splicing variants A, B, C, and D have been deposited to GenBank under Accession Nos. AF367202, AF411579, AF395908, AF395909, AF411577, and AF411578, respectively.

*Detection of ABCC11 and ABCC12 transcripts in human normal tissues and cancer cell lines.* Transcripts of ABCC11 and ABCC12 genes were detected by means of PCR, where human cDNA of normal tissues and cancer cell lines were purchased from Clontech. The PCR primers to detect ABCC11 and ABCC12 were as follows: C11 forward primer: 5′-TCTGCGA-CCTTCTTGTTTGG-3′, C11 backward primer: 5′-TCAGTACAGCATTTGCAACACTT-3′ and C12 forward primer: 5′-CACCGCCTCTATGGACTCCAAGACTG-3′, C12 backward primer: 5′-TCAATCTCAGGCACTGGGGTGGT-3′. The PCR consisted of 38 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 30 s and was followed by reaction at 72°C for 2 min.

## RESULTS AND DISCUSSION

### *ABCC11 and ABCC12 Genes Located on Human Chromosome 16q12*

Two new ABCC transporters, named ABCC11 and ABCC12, were identified by database search on human chromosome 16 working draft (GenBank Accession No. AC007600) using the BLASTN program. In the present study, we have cloned cDNAs of these two new ABC transporters and their splicing variants to analyze the genetic polymorphism and expression profiles.

ABCC11 and ABCC12 genes are tandemly located on human chromosome 16q12.1 in a tail-to-head orientation with a separation distance of about 20 kb (Fig. 1). The ABCC11 gene is encoded by a ~68 kb gene consisting of 30 exons, whereas the ABCC12 gene spans a ~63 kb size and consists of 29 exons. The cDNAs of both ABCC11 and ABCC12 had a Kozak consensus initiation sequence for translation (26) around the first ATG region, namely, 5′-CTG**A**AA ATG **A**-3′ for ABCC11 and 5′-ATC**A**GG-ATG **G**-3′ for ABCC12. The amino acid sequence deduced from the cDNA sequence with the GENSCAN program revealed that ABCC11 and ABCC12 cDNAs contain single open reading frames encoding proteins consisting of 1383 and 1359 amino acid residues, respectively. ABCC11 and ABCC12 proteins have two sets of Walker A and Walker B motifs as well as two ABC signature sequences, so-called "C motifs," within the deduced protein. In terms of the amino acid sequence, the identity of ABCC11 with human ABCC1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 was 30.7, 30.8, 30.9, 32.9, 40.1, 29.9, 26.0, 27.8, 27.9, and 29.3%, respectively. Likewise, the identity of ABCC12 with human ABCC1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 was 31.1, 30.4, 30.0, 32.8, 43.6, 28.8, 26.9, 27.9, 27.8, and 29.0%, respectively. The identity between ABCC11 and ABCC12 was 47.4%. Based on the phylogenetic relationship, ABCC11 and ABCC12 are suggested to comprise a new subgroup with a close relation to ABCC5 that reportedly transports several organic anions, including nucleotide analogues and cyclic nucleotides (23, 27, 28).

### *Splicing Variants of Human ABCC11 and ABCC12*

Figure 2 shows splicing variants of ABCC11 and ABCC12 cloned in this study. The cDNA of ABCC11 variant A consists of 4476 nucleotides with 29 exons; however, the exon 28 is entirely deleted. This intron splicing follows the conventional GT-AG rule. The cDNA of this variant encodes a protein consisting of

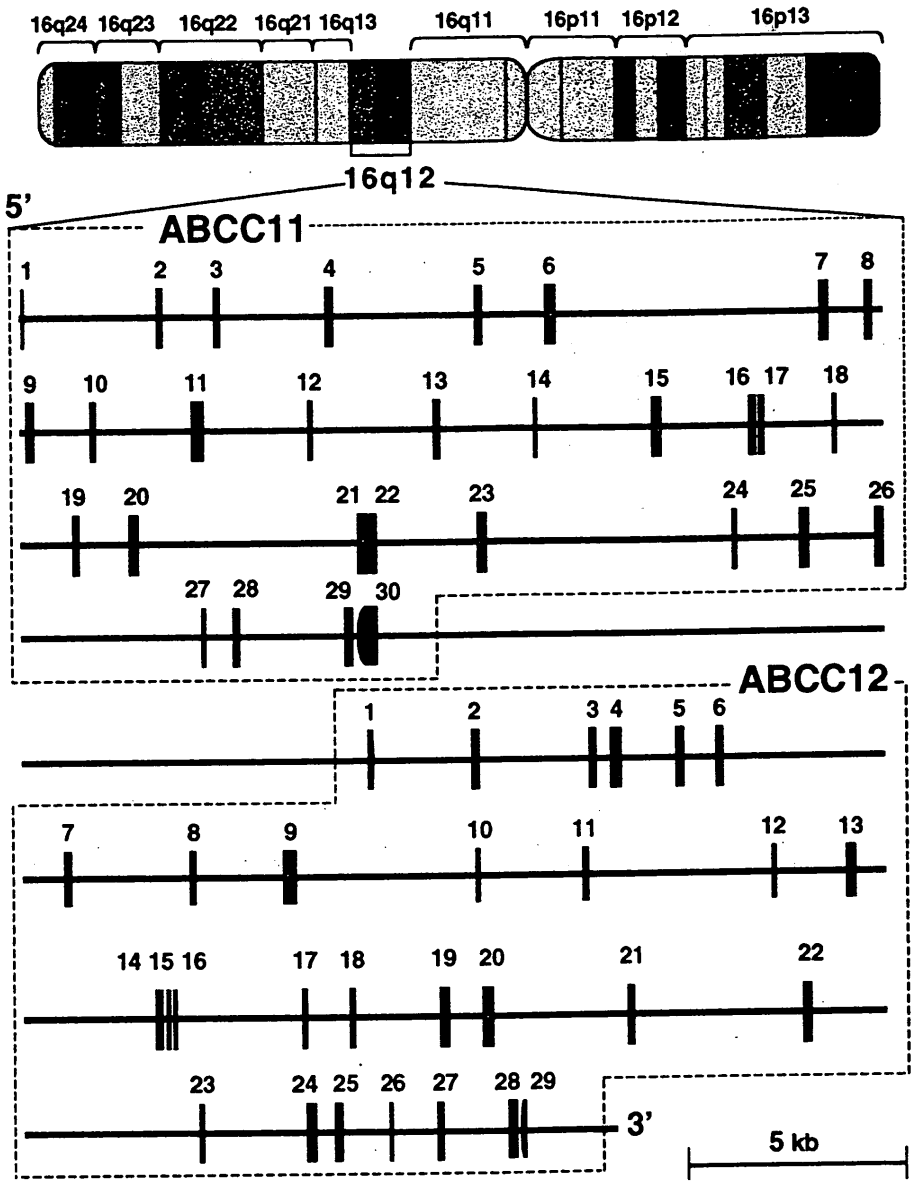
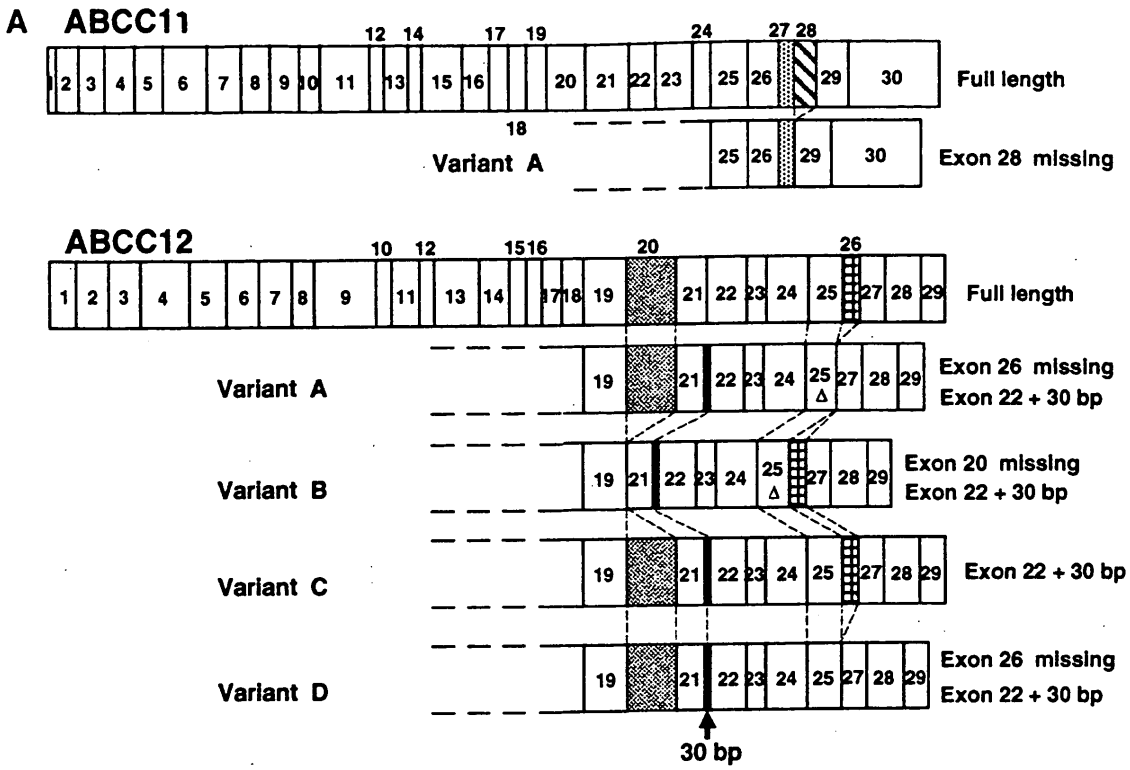



**FIG. 1.** The genomic structures of ABCC11 and ABCC12 genes on the human chromosome 16. The cytogenetic location of the ABCC11 and ABCC12 genes as well as the structures of exons and introns were analyzed by BLAST search on the Human Genome Project Working Draft (http://genome.cse.ucsc.edu/).

1344 amino acid residues. Based on hydropathy analysis, it is suggested that the variant A has 12 membrane-spanning domains like ABCC11 (Fig. 3, left). However, due to the deletion of the exon 28, the variant A protein lacks 38 amino acid residues in the second ATP-binding cassette.

In addition to ABCC12, there are four splicing variants of ABCC12, namely variants A, B, C, and D consisting of 4034, 3886, 4127 and 4048 nucleotides, respectively. These splicing variants were identified in cDNA libraries from various tissues, such as adult liver, pancreas, testis, and fetal thymus. Figure 2A shows the exon alignments of the splicing variants. The 5'-half (exons 1 to 19) of cDNAs of these variants is identical to that of the ABCC12 cDNA. However, in the 3'-half, variants A and D lack the exon 26, whereas the variant B lacks the exon 20. Furthermore, both variants A and B lack 14 bp (GTAGGTACAGTAAG) in the exon 25, as indicated by 25Δ in Figs. 2A and 2B. The alternative splicing causing the 14-bp deletion may be related to the repeated GTAG sequence at the boundary between the exon 25 and the following intron (Fig. 2B). Importantly, all of these variants have an extra 30 bp sequence at the 5'-end of the exon 22 (Fig. 2A), where a putative stop codon for translation, i.e., TAG or TAA, was incorporated in their cDNAs (Fig. 2B).

Figure 3 shows the putative protein structures of these splicing variants. Variants A, B and D cDNA



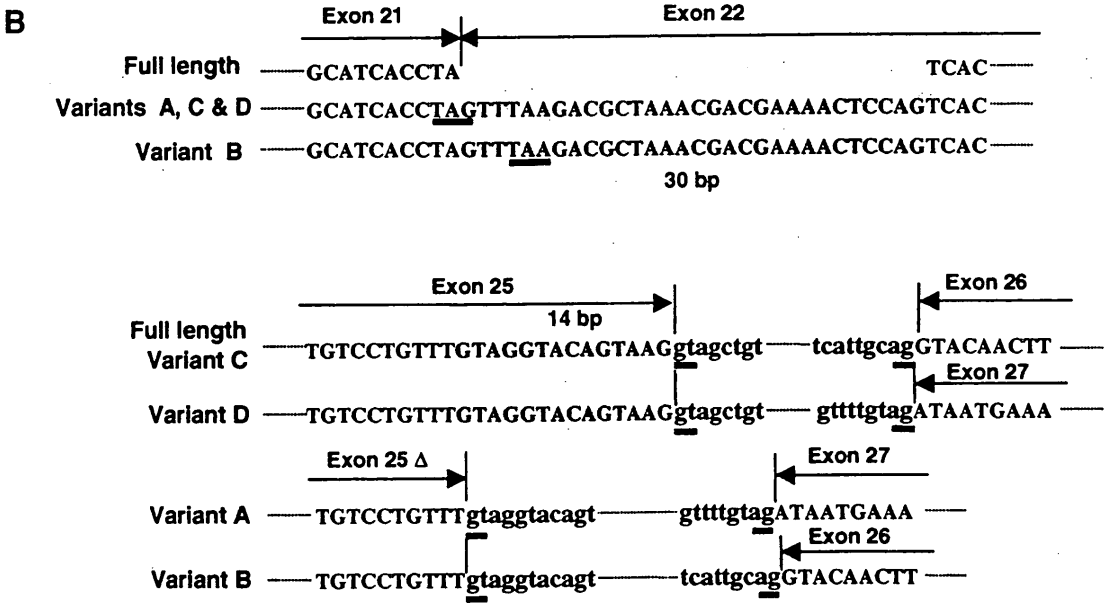




**FIG. 2.** (A) Schematic illustration of the cDNA structures of ABCC11, ABCC12 and their splicing variants. Based on our cDNA sequence data, exon structures were analyzed using the BLASTN program (http://www.ncbi.nlm.nih.gov/BLAST/) and human genome database. The number of 25Δ indicates the exon that is 14 bp shorter than the exon 25. (B) Comparison of exon 21 and 22 structures among cDNAs of ABCC12 and its splicing variants (upper column). Putative stop codons, i.e., TAG and TAA, in the cDNA of ABCC12 splicing variants are indicated by an underline. The sequence difference between the exons 25 and 25Δ of ABCC12 cDNA (lower column). The sequences of exon and intron are written in capital and small letters, respectively.

contain a single open-reading frame encoding 1009, 935, and 1009 amino acid residue proteins, respectively. Because of the above-mentioned stop codon, these variants may have only eight to nine transmembrane domains and lack the C-terminal domain with the second ATP-binding cassette (Fig. 3, right). Inter-

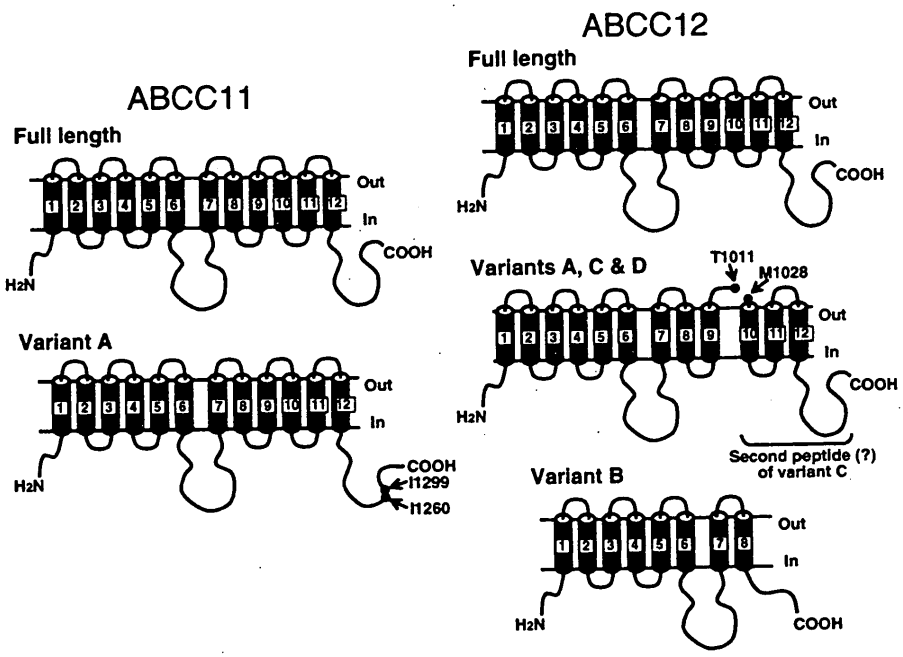



**FIG. 3.** Schematic illustration of the putative protein topologies of ABCC11 and ABCC12 as well as their splicing variants. Transmembrane domains were predicted using the SOSUI program (http://sosui.proteome.bio.tuat.ac.jp/sosuimenu0.html) and are numerically indicated in the illustration.

tingly, the cDNA of the variant C is suggested to ave two open reading frames encoding peptides consisting of 1009 and 331 amino acid residues, since the Kozak consensus sequence resides around the first ATG regions of these two peptide-coding sequences, i.e., 5′-ATCAGG[ATG]G-3′ for the first peptide; 5′-CTGAGA[ATG]G-3′ for the second peptide. Hydropathy analysis suggests that, if translated, the first and the second peptides may have nine and three putative transmembrane domains, respectively (Fig. 3). In the case of ABCC8 (SUR1), coexpression of two parts of the protein divided at Pro1042 between transmembrane domains reportedly restored glibenclamide-binding activity (29). Furthermore, it was also reported that small carboxyl-terminal deletions of up to 23 amino acids left the functional activity of ABCB1 (MDR1/P-glycoprotein) (30). Therefore, it is of interest to know whether some of these splicing variants of ABCC12 represent biological functions. Expression of those splicing variants and their function remain to be elucidated.

### *Detection of ABCC11 and ABCC12 Transcripts in Human Normal Tissues and Cancer Cell Lines*

The transcripts of ABCC11 and ABCC12 genes were widely detected by PCR in various adult human tis-es, including liver, lung, and kidney, as well as in several fetal tissues (Fig. 4). In addition, the transcripts of ABCC11 and ABCC12 genes were observed in cell lines of carcinoma and adenocarcinoma originated from breast, lung, colon and prostate. It should be noted, however, that the PCR products relatively reflected the amount of the transcripts of both full-length forms and splicing variants.

To clarify this ambiguity, we therefore cloned 30 ABCC11 cDNAs from human adult liver. The splicing variant A lacking the exon 28 (Fig. 2A) was observed at a frequency rate of about 25% in our cDNA clones of the ABCC11 gene (data not shown). Likewise, we have cloned a total of fifty cDNAs of the ABCC12 gene from adult human liver, testis, and pancreas, as well as from fetal liver and thymus. Interestingly, cDNAs of splicing variants A, B, C, and D (Fig. 2) were predominant, exceeding full-length one. Indeed, the total of those four splicing variants was more than 95% of the cloned cDNAs (data not shown).

## CONCLUDING REMARKS

The present study provides evidence that ABCC11 and ABCC12 genes are transcribed in multiple splicing variant forms. Detailed profiling and functional analysis of these splicing variants needs further studies. During this study, Tammur *et al.* have most recently reported the cloning of ABCC11 and ABCC12 (31), however splicing variants of these genes were not addressed in their report. On the other hand, recent studies have suggested a relationship between paroxysmal kinesigenic choreoathetosis and a certain gene(s) located in the region of 16p11.2-q12.1 (32, 33).

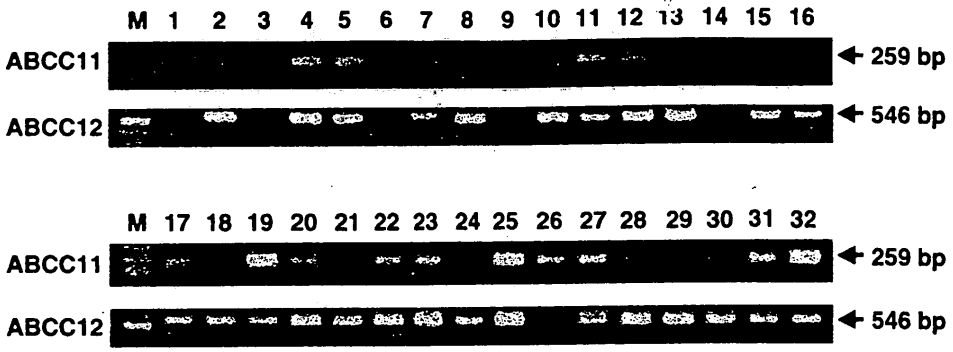



**FIG. 4.** Detection of the transcripts of ABCC11 and ABCC12 genes in human normal tissues and cancer cells by PCR. M, marker lane (100 bp DNA ladder). Normal tissues: Lanes—1, heart; 2, brain; 3, placenta; 4, lung; 5, liver; 6, skeletal muscle; 7, kidney; 8, pancreas; 9, spleen; 10, thymus; 11, prostate; 12, testis; 13, ovary; 14, small intestine; 15, colon; 16, leukocyte; 17, fetal brain; 18, fetal lung; 19, fetal liver; 20, fetal kidney; 21, fetal heart; 22, fetal spleen; 23, fetal thymus; 24, fetal skeletal muscle. Human cancer cell lines: Lanes—25, breast carcinoma (GI-101); 26, lung carcinoma (LX-1); 27, colon adenocarcinoma (CX-1); 28, lung carcinoma (GI-117); 29, prostatic adenocarcinoma (PC3); 30, colon adenocarcinoma (GI-112); 31, ovarian carcinoma (GI-102); 32, pancreas adenocarcinoma (GI-103).

Since ABCC11 and ABCC12 genes are encoded at 16q12.1, it is tempting to study the biological function of ABCC11 and ABCC12 as well as to examine a potential link between the genetic polymorphism of these ABC transporters, including multiple splicing variants, and the pathogenesis of paroxysmal kinesigenic choreoathetosis.

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